

DEVELOPMENT OF A BEHAVIORAL TECHNIQUE TO ANALYZE
A DOG'S ABILITY TO DISCRIMINATE FLAVORS IN
COMMERCIALY CANNED DOG FOODS

by

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INTRODUCTION

The word flavor is very meaningful to each of us and yet it becomes quite nebulous when one tries to describe a particular flavor. Flavor identification includes utilization of two chemical senses, olfaction (sense of smell) and gustation (sense of taste). It is the first, olfaction, that was chosen for use in this study.

For several years, dog food companies have labelled their products to specify specific flavor ingredients, such as liver, beef, chicken, or fish flavor. A question was raised by food control officials as to the validity of such labelling. Can a dog distinguish the food in a can marked liver flavor from one marked chicken flavor? This is basically the question we were to answer, though the answer included the discrimination of four flavorants.*

To answer this question, it was necessary to develop a technique by which a line of communication could be established between the canine investigators and their human counterparts, for it was the dog who was doing the work, the results being recorded by the human.

Since olfactory acuity in the dog is extremely high, we selected this factor, odor, to use as the basic criteria for discrimination. The dogs had to be trained to identify a particular odor and then relate to us their recognition of this odor. The animals had to do this in an environment free of distraction, and free from cues given by the handler. Since humans give arbitrary names to everything, including flavors, it was necessary to attempt to condition the dogs to flavors as we know

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them and then to interpret the results. Thus, the question was answered as to whether dogs can or cannot distinguish the flavors that humans have taken the liberty of marking on the food products.

MATERIALS AND METHODS

Test Foods

Since this work was sponsored in part by the Pet Food Institute, Chicago, Illinois, it was essential that the dog food used was in no way specifically produced for this research by the companies involved. It was, therefore, decided that food should be purchased in local supermarkets to guarantee the identity of test products with those purchased by the consumer. Only canned food was tested.

The test food used included all brands that produce foods containing a flavor ingredient as well as a non-flavored diet available in this area. The basic diet canned food provided a neutral base with which to compare the flavored products. One brand was included because it provided pure canned meats (beef, liver and chicken) that were used for conditioning and reinforcement. The flavors used were beef, chicken, liver, and fish. Each brand was given a Roman numeral code number which was placed on the can after removal of the label (Fig. I)*. If the manufacturer's label indicated a specific ingredient (flavor) as being present, this was identified by printing on the can, in capital letters, L for liver, B for beef, F for Fish, and C for chicken, and O for no labelled flavor. Ten different brands were used and a variety of 22 products (Table I)*.

It was assumed that when a can was labelled chicken flavor, the chicken in the can was the meaty protein and not the entrails or feathers. The amount of flavorant was also unknown. Ingredients, as listed on the

*All figures and tables in Appendix.

label, were the only information available; commercial companies do not reveal their food formulas.

The food used for experiments was at room temperature and opened just prior to its use. Both temperature and age, which alter the flavor contents, had to be kept constant throughout the series of trials. The food sample of approximately one tablespoon was presented to the dog on plastic-coated meat trays. New trays were used for each trial so that no residual odor remained.

Vegetable dyes were used in a few trials to alter the foods appearance and determine the importance of color discrimination by the experimental subjects. This was especially important to the chicken conditioned dogs for their positive food was usually a pale color.

Subjects

Eight dogs were used for the experiment, a male and a female for each of the four flavors. The dogs, though kept in kennels, were treated as pets. The two chicken conditioned dogs were Beagles from the university kennels. Big'n, a male Beagle, was worked initially on method #2 and then #4. He was conditioned to chicken during previous experiments. Roan, an old female Beagle, worked on #3 and #4. The beef conditioned dogs were Mickey and Brownie. Mickey was a standard Poodle house pet that started training on method #1, and then was switched to chicken for method #2. She was reconditioned to beef for method #3 and #4. Brownie, a university male Beagle, was previously conditioned to beef and worked on methods #3 and #4. Spike and Bert were the two fish conditioned dogs. Spike, an English Pointer male, was a privately owned hunting dog who was conditioned for methods #3 and #4. His counterpart, Bert, was a female

university Beagle also conditioned for methods #3 and #4. The liver testing dogs were Scarface, a university male Beagle, and Lady, a Brittany Spaniel female, obtained from the pound. Both of these animals were conditioned for methods #3 and #4.

The maintenance diet consisted of dry commercial dog food mixed with the negative samples, unconditioned stimulus (U.C.S.), from the day's trials, so that outside the test area, dogs ate a variety of flavors.

Problem Box

The box described below was used for all methods with modifications listed later. The test box consisted of a three-sided reinforced masonite enclosure, 4 feet by 4 feet per side. The back was left open and placed against a wall; no top was put on the test area. The three panels were hinged so that the entire unit could be collapsed for convenience and ease of handling and storage.

The front panel contained Mirropane (a two-way mirror) mounted in the center so that the dogs could be viewed during the trials and records taken. A high intensity light was placed in the box, behind the dog so that it illuminated the mirror and the dog saw its reflection rather than viewing those observing him. Throughout the experiments, the dogs gave no indication of being able to see through the glass.

Below the mirror was the drawer designed to deliver food samples to the test animals. For method #1, the drawer held one meat tray. It was enlarged to accommodate two trays for methods #2 and #3 and enlarged to six trays for method #4.

A small opening, 3 inches by 5 inches, was made on either side of the mirror for reward delivery in method #1. Swinging doors were placed in this same position for reasons described in method #2. The openings were sealed and not used for methods #3 and #4. Figures II and III illustrate the box as it appeared for method #3, which is the same for #4 except that the retractable drawer was increased to hold six trays.

Experimental Techniques

When initiating the work, several methods were tried while attempting to find the method suited to the problem. It was necessary to condition a dog, develop a communication system to indicate the dog's acknowledgment of flavors to which it had been conditioned and accomplish both with expediency. Methods #1 and #2 will be discussed briefly as preliminary methods and #3 and #4, which were of primary importance, will be covered in greater detail.

Method #1

The object of this method was to condition the dog to a flavor, stimulating the dog to respond by putting its nose through a small opening to its right and receive a reward, a semi-soft commercial treat. When this was accomplished, a second flavor was introduced, to which the dog was to go to an opening at its left for the reward.

Method #2

It was decided that the dog would be less confused if required to recognize only one meat flavor but show a more definite response upon recognition of that flavor.

The sample drawer was enlarged to hold two trays, and doors were put in the box from which the dog could exit to receive a reward for choosing the correct sample.

To begin conditioning, the dog was placed in the box and fed the ingredient to which it would be conditioned. Upon consumption of the sample, the dog was coaxed out of the problem box and given a reward of the same product to which it was to be conditioned.

Once the dog responded readily to the first phase, it was introduced to two products simultaneously. One product was the conditioning flavor, the other a product with another flavor or one for which no flavor designation was given. If the dog ate the conditioned stimulus (CS) flavor to which it had been conditioned, it was allowed out of the box to receive a reward. If it ate the other sample, the dog had to remain in the test box. The dog soon learned to disregard the alternate sample and consistently chose the CS.

Method #3

Neither methods #1 nor #2 produced satisfactory results so another method was tried. This technique was by far the most successful and can be broken down into three phases.

First was the habituation-training phase which was a conditioning period without challenging flavors. The dog was placed in the problem box and, by the use of the drawer and plastic-coated meat trays, was fed the flavor he was to be conditioned to recognize. All dogs but two were presented with the natural, pure product, either canned chicken, liver, or beef. The dogs conditioned to fish were conditioned to a fish-flavored commercial dog food because no pure variety fish product was available.

As soon as the dog showed no hesitance in approaching the tray and eating the conditioning flavor, phase two was started.

The conditioning phase, phase two, consisted of the presentation of the conditioning flavor and the selected commercial dog food products containing no labelled flavor, as brand V-O, or a commercial product containing a labelled flavor, as V-L. The flavored food presented was, of course, not the same as the conditioned stimulus to the dog. Several brands or flavors were used for challenge flavors each day so that the dog was working for a CS rather than against a UCS. If the dog selected the natural product or that specific flavor to which he was to be conditioned, he was permitted to eat the food from the tray. Eating the food was considered a taste reinforcement as well as a reward for having made the proper choice. If the dog selected the unconditioned stimulus, negative food sample, the drawer was quickly retracted so that the subject was unable to eat the sample, thus receiving negative reinforcement.

Further negative reinforcements were tried on the first dog to undergo this method and were found to be too harsh. Those used were a voice command, "No", and an air jet mounted over the negative sample so that if the dog made an attempt to eat it, a stream of air was released to induce avoidance. Not only did the dog avoid the negative sample, but refused to eat from the tray entirely. This method was quickly eliminated in favor of the simple retraction.

Each conditioning or test session consisted of 10 - 20 presentations. As each dog worked individually, a session was limited to the individual appetite of the dog. The dogs conditioned rapidly and were considered conditioned when one session (10 - 20 trials) was completed without error and the dogs were obviously working to make a correct choice.

The testing and reinforcement phase consisted of the presentation of the CS in either the pure form or the flavored dog food in one tray and the base product or a product with another flavor in the other tray. The trays were randomly placed in the drawer so that no pattern could be followed in the selection of the food. Each CS, pure and flavor, was tried against the complete list of challenge products. The foods were presented as rapidly as possible, depending upon how quickly the animals responded. Here, too, the number of trials per session was regulated by the individual dog's appetite on a given day, but the usual number was 10 - 15 presentations (Table II and Figures II and III).

Method #4

This method was simply an extension of Method #3, with only experimentally sophisticated dogs used. Those conditioned to Methods #2 or #3 were given the additional challenge of differentiating their specific CS from among five other flavorants or bases. The positioning was random and the challenging flavors and bases were presented with the CS (Table III).

The number of samples was increased to the maximum in the first trial, an increase from two to six samples. No gradual increase seemed necessary. Figures IV - VIII show a dog being tested with this method.

LITERATURE REVIEW

Part I - Olfactory Mechanism

Though the process of olfaction had undergone many years of scrutiny, it was amazing to note with what regularity the introduction of current literature began by stating how little is known about the field of olfaction. As Biedler (1954) stated, "This is a virgin field."

Though much has been done in this field in the past fifteen years, little has been accomplished to clarify understanding of this field. In fact, it has only been confused by the addition of many seemingly well based, but conflicting reports.

The classical anatomic description of the olfactory mucosa is well known. Yet there is still some discrepancy as to the exact structure of each cell. The bipolar receptor cell, olfactory cell, is classically described as having an apical knob or rod from which extend thousands of hairs, 1-2 microns in length (Guyton, 1961, p. 740 and Adrey, 1959). This contrasts to Gasser (1956) who described five to six cilia, 100 in length, each of which break out into lateral streamers. De Lorenzo (1963) who described six to twelve cilia and Clark (1957) who described nine to sixteen hairs, stated nothing more of a finer structure. It was agreed that this end of the cell was the only free end. De Lorenzo (1963) also described vacuoles in the rod area indicative of active pinocytosis. If this was the case, there is a correlation between these vacuoles and one of the mechanisms of olfaction to be described later.

Continuing along the body of the cell, it was found that sustentacular cells create a sheath around the olfactory cell. Glasser (1956) stated that the basal cell made up the first part of the nerve fiber

sheath. Once through this layer, the fibers are then sheathed by the Schwann cell. These individual fibers, in the ratio of one fiber per receptor, were estimated to be 2 microns in diameter and converged to form mesaxons, which then form fascicles. These continued to the olfactory bulb at which point the classical description again takes over.

One of the first men to work on the electrophysiological mechanisms of olfaction was Adrian (1951, 1955, 1957). He attributed odor selectivity to individual receptors. One type of receptor would transmit electrical potential for one group of odors, another type for a different odor. He stated that these receptors were located in groups with different locations for different receptors or there was an intermixing of receptor types within one location. Adrian's work was concerned with the quality of odor and not the concentration or odor thresholds. Gesteland et al., (1965), concurred with the earlier works of Adrian.

Mozell (1964) continued in this area and produced evidence for the sorption hypothesis as a mechanism for odor analysis by the olfactory region. He stated, "the receptor sheet as a whole might separate chemical vapors by adsorption or absorption in a manner analogous to gas chromatography columns." There is a neural encoding of the analysis via a space-time sequence, that led to the Spatio-Temporal Patterning idea of odor recording. This pattern was indicated by discharges in the olfactory bulb as differentiating spike activity. Mozell (1958) used anesthetized rabbits, but Moulton (1963) whose subjects were also rabbits, used chronic electrode implants for his recordings. Both found a definite pattern related to a specific odor. The variation in the spike activity involved latency, duration, rate of growth, and decay of response. There was a variation seen in anterior and posterior parts of

of the bulb, but none vertically. Concentration did not alter the response; however, the response increments decreased as concentration increased. The asymptote was reached at a much lower concentration level than was seen in the other senses. In olfaction, the asymptote occurred at 10 - 15 times the threshold concentration, whereas the asymptote in other senses was reached only after the concentration was increased several thousand times. One discrepancy in the spatio-temporal system is that there were not enough patterns for the many varieties of odors. It may act as a sorting out mechanism.

Hughes and Hendrix (1967) added to the above with the frequency component hypothesis which stated that odors of different stereochemical categories vary in regard to all the frequency components seen in the response. The main signature of a given type of odor will be seen in the peaks of highest amplitude in the response, but minor peaks of lower amplitude will also help specify the nature of the stimulus odor.

Benjamin (1965) stated that current work showed an inhibitory mechanism in the bulb which was a result of several feed-back loops. This involved fibers of the central nervous system as well as fibers in the bulb continuing to the level of the glomerulus.

Some of the basic work on olfaction at the cortical and subcortical level was done by Swann (1933), Allen (1938, 1940, 1941), and Brown and Ghiselle (1938). Their work was done on dogs and rats conditioned to perform various assignments after presentation of a specific odor stimulus. Lesions were then produced in various areas and the general conclusion was that odor identification and learning efficiency was not impaired by destruction of the cortical or subcortical areas. Apparently, these areas are involved in secondary responses, such as sexual stimulation

or hunger, as a result of odor stimulation and identification. Later work by Boudreau (1964) was done with cats by placing chronic electrodes and submitting E.E.G. recordings to computer analysis.

Thus far, the olfactory nerve has been covered quite extensively and little is known about its involvements. The trigeminal nerve is even less understood. As stated by Tucker (1961), it was once thought that only high concentrations of odors would trigger a response, but later findings showed that this was not always the case, as different chemicals brought about a different response. The response, at times, was faster than the response by the olfactory fibers. The trigeminal may have greater significance in lower animals.

The Vomeronasal organ or Organ of Jacobson may also serve as an addendum to the olfactory area. Though it is a vestigial organ in man, it is located in the more anterior aspect of the nasal cavity and may have some olfactory ability. The receptors of the organ appear to be the same as those in the olfactory region.

Part II - Odorants and Flavors

To be smelled, a material must have certain properties. Parker and Stabler (1913) stated that such materials must be vaporous or gaseous. They also stated that odorous particles are dissolved by nasal secretions and then activate receptors. It is commonly understood among authors (Guyton, 1961, p. 740) that odors must be volatile to be inhaled and slightly water soluble to penetrate the mucous covering of the olfactory area. They should be lipid soluble since the olfactory cell membrane contains some lipid. This would be necessary only to promoters of certain theories in which the odorous material passes the cell membrane.

The classic taste factors are listed by Guyton (1961), p. 735, as salt, sweet, bitter, and sour. This classification has long been accepted, yet there are no such accepted classifications used for odor identification, though one is coming into prominence. Jones (1957) listed four factors to be enlarged upon after future studies. These factors were the following: I. pyridine and n-butyric acid, II. isomeric butanols, III. ethyl acetate and ethyl chloride, IV. n-caprylic acid and amyl acetate. Jones saw no correlation between chemical dimensions and homogenous odor qualities, but attributed odors with their ability to active receptor sites. The latter is in agreement with Adrian's theories of olfaction.

The findings of Jones (1957) contradicted Amoore (1964) and Amoore and Venstrom (1967) who cited a definite correlation between molecular structure and odors. Adrey (1959) cited Hill and Carathers for their statement that the number of atoms in macrocyclic ring hydrocarbon compounds is related to the nature of their odors.

More dramatic proof, and much more accepted, is the stereochemical theory of odor, Amoore (1964), Amoore et al. (1964), Amoore and Venstrom (1967). This classification of odors is listed as ethereal, camphoraceous, musky, floral, and minty. Putrid and pungent are also listed but these odors are due to the chemical charge of such compounds and not to the stereochemistry. These investigators have gone to great length to show the definite configuration of the various molecules. Each shape has a coexisting receptor area on the receptor cells. These "slots" were described to the exact dimension in Angstrom units to show width, length, and depth. No further proposal was made as to how the identification of these molecules was transmitted to the brain for classification.

Another characteristic of odorants as cited by Wright and Michels (1964) and Demerdache and Wright (1967) is the far infra-red vibrations yielded by each molecule. Each odor was said to have had a low frequency molecular vibration. This, then, affected the receptor cells of the olfactory mucosa. Much controversy is present about this theory with many in complete opposition, but Briggs and Duncan (1961) said that the carotenoids of the olfactory mucosa were responsible for the absorption of the infra-red emitted by the odorants and that animals without the carotenoids were anosmic. They went on to say that since the carotenoids cannot be synthesized by animals, they treated primary anosmia by injecting Vitamin A. It was not stated, however, in what manner they detected the fact that these cattle were unable to smell.

The odor mechanism of pure chemicals is far from solved, and yet industry is pressing for knowledge in the field of flavor chemistry. Why does a product smell and taste as it does, and how can this be duplicated? Let us consider the problem of meat flavors. Hornstien et al. (1960) studied the actual composition of meat. Beef extract was made from raw beef and analyzed for carbonyls and acidic and basic components. Good reproduction of the extract was obtained by using the longissimus dorsi muscle. It was the water extract that contained the flavor and yet this flavor will be altered when fat is added. Patton, et al. (1966) concurred by stating that solubility in water or fat alters flavor. The change is proportional to the degree or percent of fat and water that is used. Artificial flavors may be produced by instrumentation, but are usually detectable as being artificial (Patton, et al., 1966).

When studying flavor or odor, most of the work has been done by human subjects. Miller and Erickson (1966) felt that in flavor discrimination tests which utilized animals, the experiment should be designed to utilize the sense of olfaction. If taste was to be the criteria, then the olfactory mechanism should be eliminated. Rats used for taste experiments were found to be utilizing their olfactory ability instead of gustation. Jones (1955) compared techniques in smelling and concluded that sniffing, though 80% consistent, was not as controlled as blasting. In sniffing, the subject sniffs the odorant; in blasting, a regulated amount of air is forced into the subject's nostrils. Though the latter may be more controlled, the former was found to yield more acute results.

It must be kept in mind that when animals are used for testing, sniffing is the only reasonable means of attempting olfactory identification.

Part III - Conditioning Techniques Used in Olfactory Experimentation

Techniques for olfactory research in animals are rare, due to cost and length of time required to condition the animals. The rat is the most commonly used animal. A few researchers have used the dog as an experimental subject for this type of research. Other than Russian workers, whose reports are incomplete (Becker, et al., 1962), Allen (1937) was one of the first men to associate conditioning methods with olfactory research. He successfully conditioned dogs to use the forelimb response when clove or asafetida odor was presented to them. When water blanks were presented, the dog gave no response. Allen then found that the dogs showed a forelimb response when a second odor was presented.

The subjects finally learned not to lift the forelimb to other odorants. This long process of learning allowed for discrimination of odorants, but Allen did not mention the possibility of cuing. Thus because of the time element, cuing and the fact that negative conditioning was used this method was considered undesirable.

Becker et al. (1957) were the first researchers to try to use dogs objectively in olfactory research. To interpret an animal's response to an odor stimulus, it had to be made certain "that the animal was responding to the test odor and not some other identifying odor quality, and that the animal was reacting by smelling and was not responding to some other total complex which might be operating in the experimental situation." The first problem box was designed so that the dog was presented with two odorants and upon choosing the positive, could turn in the direction in which the positive sample was positioned and obtain a reward. Their first problem box presented several difficulties. First, the odor stimulants came via gas vents in the floor which the dogs would neglect to sniff. This was later changed to plates, but they were still on the floor. Second, they used both a positive and a negative reward method and this seemed too complex; the food reward use was also too distant. Third, they used clove or anise in paraffin versus paraffin or asafetida and found the concentration of odorants was too high for effective discrimination. Fourth, they tried using a light over the positive odor during the training period and soon found the dogs cuing on the light and not bothering to sniff. Fifth, the time required to train the dogs was unreasonably long and good results never were accomplished.

The second problem box was of simpler design. The box was painted so that one half was white, the other black. The odorant samples were placed on a shelf for easy accessibility to the dogs and the food reward was brought closer, with the negative reward being eliminated entirely. The object was to have the dog enter the box, sniff the sample, and if positive, go to the color side it had been conditioned to associate with the odor for a food reward. Should the odor be negative, the dog had to go to the color it was trained to associate with a negative odor sample. The odorants (Becker et al., 1957) used were clove versus paraffin and beef bouillon versus water. It was found that the dogs worked quite well for the significant odor of food (beef bouillon) but diminished in performance for the clove odor. This box was also unsatisfactory for the animal had to be handled and some cues were inadvertently being given by the handler. The results obtained were also unsatisfactory and again only little above chance.

The third problem box was designed to eliminate contact with a handler. The dog entered a narrow center aisle and walked to the odor sample which was contained in a funnel at approximately "nose" level of the dog. Two seconds after the dog sniffed the funnel, a buzzer sounded and the dog was allowed to enter a chamber containing food reward. This box was painted half white and half black as was box number two, with half of the dogs going to the black side on positive stimulation and the other half going to the white. It had been decided that the dogs would have to work at a criterion of 80 percent efficiency but only six reached this level and then only after several hundred trials. When the dogs did work well, it was only for a period of three to four days, so

that chance results were still often obtained. This defect also led to the elimination of this method for threshold determination studies.

This same group of researchers, Becker et al. (1962) later tried an entirely different method for threshold studies. The apparatus consisted of a circular table with five equally spaced stations. This table was in a large room which had two anterooms, one for the recorder and one for the handler and dog. The dog and its handler remained in the anteroom while the samples were being placed on the table. They then approached the table, the dog being allowed to sniff the samples at random. The dog was conditioned to sit to receive a food reward when it came to the station containing the positive sample. The dogs usually made one complete trip around the table and if the sample was missed, would proceed around a second time. If the dog showed no recognition at that time, the dog was removed to the anteroom to await repositioning of the samples. The handler was not aware of the correct position, since he was with the dog, but the paper never stated how the handler knew to give the dog a reward when it sat at a station.

The concentration of samples was lowered until the dog worked only at chance ability. It was found that the dog's acuity was greater if the concentrations were lowered in gradients as they were presented to the dog. If the samples randomly varied in concentration from trial to trial, the dog was unable to differentiate the lower concentrations it had been able to discriminate when the gradual decline was used. It was also stated that experienced dogs worked better than experimentally naive dogs.

King et al. (1964) used the above described technique for identification of fingerprints on glass slides. It was noted in this paper that two general errors were seen. The "By" in which dogs passed up the sample and were allowed to recircle the table and the "Before" error. The latter showed three forms: (1) dogs gave up before attempting identification, (2) the dogs wanted the meat reward and would sit without trying or working for the reward, and (3) position habits developed in some dogs.

Another group of researchers, Eayrs and Moulton (1960) worked with rats and found that they worked better for food odor and that cuing presented a problem. These animals worked in a circular cage and were presented with an odor; if the odor was positive the subjects were allowed a drink, if negative, they received a shock.

This same group (Moulton et al., 1960) worked with dogs on threshold studies. A three room arrangement was used, but in this experiment, the test room contained four crucibles, one in each corner, that contained the odor. The dog was taught to sit upon recognition of the odorant to receive a food reward from the handler. It is noted in this paper that the recorder would activate a small light above the sample if the dog had made the correct decision so that the handler would know whether or not he should give the dog a reward. Only two dogs were used in this experiment and each showed radically different results, so that more work was needed before conclusions could be made.

No doubt pet food companies have done work in this area, but since the material is never published, it is impossible to know what results, if any, have been accomplished.

RESULTS

Method #1

This procedure was the first attempt at conditioning a dog to a flavorant. Mickey, used for this study, was worked for 479 trials at approximately 12 - 15 trials per session. She was first taught to go for her reward to the right when beef was presented. This was accomplished after a short period of time; she was then alternately presented with chicken, to which she was to respond by going to the opening at her left for a reward. After several unsuccessful trials, she was presented with only chicken and taught to go to the left. Upon obtaining an efficiency of approximately 80 percent she was presented with beef intermittently with chicken. Again, she reacted with confusion and had to be coaxed to make most of her decisions. This experiment continued until it was obvious that Mickey was trying to "out-guess" the researcher rather than attempting recognition of the CS. She would quickly go from one opening to the other in an attempt to gain a reward, occasionally selecting the proper one but by no means better than chance.

Method #2

Because it was felt that a definite conditioned response (CR) was necessary, the dog was required to leave the box when a choice was made to receive its reward. Big'n had been conditioned to chicken on previous experiments involving gustation. The condition obviously carried through to olfaction since it was only necessary to condition him to the proper response upon receipt of the CS. A challenge flavor or base was presented with the CS and Big'n made only 11 errors during 695 trials.

Admittedly, this is an exceedingly high number of trials but Big'n was the first dog conditioned to a flavor successfully and it was necessary to prevent any degree of extinction.

In one session, he was presented with a challenge flavor that had been kept open and at refrigeration temperatures over night. He worked with hesitance, but did make the correct choices. The primary difficulty he had was in recognizing VII-C. He would sniff both samples several times but make the right decision. When VII-C was challenged with VII-L, he refused to eat either sample and tried to come out of the problem box without having made a decision. His primary cause for error seemed to be haste.

Big'n was eventually treated as a Method #3 dog and not allowed to leave the box for an additional reward. This didn't seem to disturb his momentum, but he would often try the door to see if it would open.

An attempt was made to condition Mickey to this method but was abandoned after 259 trials. She had no desire to leave the box via the door provided, but would sit before the delivery drawer and wait for the next sample delivery.

Method #3

This method was by far the most satisfactory and was adopted after a definite pattern of conditioning was established with Roan. Roan, an old female Beagle, was very apprehensive in the problem box. Her habituation period was slow when compared to other dogs. Once the conditioning-challenge phase was started, rather than correcting her by withdrawal of the tray and not allowing her to eat, she was subjected to a slight jet of air. This was enough to fortify her apprehension and

she refused to eat from the meat trays for the following sessions unless strongly coaxed. A vocal "No", was also tried, and this too proved too strong for this shy animal. It was found that even the most extroverted dogs responded well to the simple withdrawal of the samples as a corrective method.

Though nervous, Roan was a very methodical worker and errored only once in 210 trials. Her CS was chicken and she, too, had trouble differentiating VII-C, especially when challenged with VII-L. After sniffing several times, she would choose the chicken. Samples were presented randomly but Roan always approached the trays from her left.

One week lapsed after Method #2 was discarded for Mickey, when she was started on Method #3. Whether or not she recalled her earlier conditioning to beef is unknown, but she responded to this method quickly and of 139 trials, errored only five times. Three of these errors were for III-B and one each for IX-B (pure beef) and VIII-B. The latter two appeared to be due to lack of concentration. III-B presented some problem of actual recognition, though she did make positive identification of it several times.

Brownie had been previously conditioned to pure beef on gustation studies. His habituation period was brief and he worked well on IX-B and VIII-B. Brownie's problem came with III-B. Of the 15 errors made during 213 trials, 14 were with III-B. On nine other trials, when presented with III-B, he refused to eat either sample. These were not considered errors because he made no attempt to eat the UCS. If the slightest amount of IX-B was smeared on III-B, there was no hesitance in his choice. A period of two months lapsed during which he was not

worked, but upon confrontation with the problem worked correctly for each choice; however, he still rejected III-B.

Scarface, a liver conditioned dog, erred in four trials out of 205. His frustrations upon indecision were obvious as he would circle and bark in the box. VII-L gave him the biggest problem. Once corrected, he refused to eat unless vocally coaxed to the front of the box. On one occasion, he remained in the box 20 minutes without attempting a decision, so was put back in his cage.

Lady was a very temperamental Brittany, conditioned to liver. There were only six obvious errors in 214 trials, but on several occasions, she rejected samples completely. She was inconsistent in her reluctance to eat samples, for one day she would choose to avoid I-L, another day II-L, and still another day IV-L. Previously or subsequently, she chose these same products as the CS.

A male pointer, Spike, worked 179 trials during which time he erred four times. Habituation came rapidly, but while on the condition-challenge phase, he required three sessions to be enlightened to the fact that the fish product was always present. If he approached on the fish side, he ate the sample and went to the back of the box to await the next delivery. If he approached the challenge side, he went to the back of the box without eating. Even though the two samples were placed side by side, he wasn't aware of the presence of the second. Once he discovered he would have a sample to eat upon each presentation, he worked well. When Spike was corrected by receiving negative reinforcement, he would lie down at the back of the box, not to be raised unless to be let out of the problem box and that session had to be terminated.

Bert was a gluttonous female Beagle. She ate with such gusto that her habituation phase was literally non-existent. The problem came during the second phase, when she would try to eat both samples before the tray could be retracted. It was finally decided that the only way to slow down this animal was to feed her prior to the experimental session. After that, she worked quite well, erring seven times in 226 trials, with six of the errors being III-F.

Method #4

In this method, each of the challenge flavors was presented at least five times with each CS.

Big'n worked 148 trials without error, however, he refused to eat VII-C when presented. Instead he would eat VI-O which, according to the list of ingredients, contained some chicken even though it was not specified as being chicken flavored. If VI-O was not present as an alternative to VII-C, he ate nothing. It should be noted that after an extinction period of two months, he was required to resume trials for guests to the University and did so without returning to Method #3 except for a single presentation of the pure chicken.

Roan also worked without fail on 134 trials. As Big'n had done, Roan ate VI-O, if present, rather than VII-C; if not, she ate nothing. Roan's extinction period lasted three months, after which she was returned to Method #4 upon the completion of one session of Method #3.

Brownie worked 113 trials with three errors. Two of these were with VIII-B which usually gave him no trouble. The third was VII-B; on two other occasions when presented with VII-B, he refused to eat it or the other samples. This was not counted as an error. Brownie was

presented with III-B but refused it as he had during Method #2.

Mickey erred five times out of 80. She refused to eat when either III-B or VII-B were presented to her.

Scarface worked 159 trials with six errors, one on XII-L and five on II-L. He showed spasmodic reactions to II-L, one time eating it, the next time not. He refused all samples of VII-L, the same product that gave him difficulties in Method #2.

Lady, also a liver conditioned dog, completely broke down when subjected to Method #4. She completed two sessions and then refused to eat. She was then returned to the double presentation and was gradually increased to three and four samples but she would never work more than four trays at a time unless the CS was IX-L (pure liver). During this period of "reconditioning" she had to be continually coaxed to approach the front of the box and sniff the samples. More often than not, she remained in a reclining position at the back of the box, even when fasted the previous day.

Spike worked without error on 96 trials. He was allowed to lapse a month and when brought back was working on six trays within one session.

Bert also worked well, but unfortunately her data for this method was lost when the animals were moved to a new facility. One session is recorded, however, and showed 17 accurate trials with all three CS flavors used in this session. It is the author's opinion that Bert responded equally well to the additional trials attempted.

DISCUSSION

Many problems arise doing research work in the field of biological science. This is intensified when the research involves animal behavior.

Eight dogs each possessing individual attitudes were used in this study. One of the most obvious differences in this group was the difference between the university kennel dogs and the personal pets. The Beagles were from the university and though each possessed a certain temperament, generally these dogs were much more responsive to kindnesses and more willing to work in the test area, eating food to which they were conditioned. Scarface, when out of the test area, preferred any flavor other than liver to which he was conditioned, and yet he would discriminate against the flavors he preferred when in the test area. Mickey and Lady acted differently. Lady wouldn't work on the multiple system except when she was given the CS of pure liver, IX-L. If she did sniff the trays, she would stand before the positive tray, but refused to eat the sample unless the handler would put his hand in the tray and let her lick the test material off of his fingers. Thus, these trials had to be discarded from any actual data. Mickey was very sensitive to her surroundings. While in the box, she would listen for voices and was easily distracted if anyone moved about. Some days she concentrated well; others she would just sit and listen and pay no attention to the trays being delivered to her. If she didn't eat in the box, she obviously knew she wouldn't starve. The kennel dogs acted as if they had better eat one of the samples presented or there wouldn't be any more. Actually, each dog was kept on a maintenance diet throughout the experiment, with weekly weight checks to keep the dogs' weights reasonably constant. The

food fed to the dogs was the negative samples not used during that day's trials. Both Roan and Bert, when released from the problem box, would try to eat samples they had definitely rejected just a few minutes earlier.

It was essential that each dog was well known and understood by the researchers. Though moods are intangible, it was obvious to those working in close proximity with the dogs when a particular dog was having an "off" day. During these times it was futile to attempt a trial. Depending on the dog, the subject would either lie at the back of the box and not move, walk around in a sort of daze, jump on the side of the box and bark, or just sit and howl. These periods were few, but did occur and should be noted. On the days they worked well, the dogs obviously made conscientious attempts to sniff all samples and made deliberate choices.

Though errors were made during the trials, they are so minimal that this data conflicts with the results of earlier workers (Becker et al., 1957). It should be noted that errors were recorded when an attempt was made to eat the UCS and the subject received negative reinforcement. More often, the dogs, not being able to recognize the CS, would simply refuse to eat either sample or samples and according to individual temperament, would sit and bay or lie down. The dog knew what not to do, he just didn't know what to do. Becker et al. (1957) stated that his dogs would not sniff, but try to go either right or left for a reward. Our dogs would sniff the samples thoroughly and reject them, not attempting to get a reward for work not done.

Correction had a great affect on the way a dog worked. As mentioned in discussing methods, various corrective measures were discarded during phase two and withdrawal of the tray was sufficient negative reinforcement. Even this at times was severe, for most of the dogs were troubled by this reinforcement and were reluctant to approach the trays after having been corrected. On occasion, the trial had to be terminated.

On what basis were dogs making choices? Because formulation of dog foods was impossible to acquire, it was assumed that the food was labelled properly. After being conditioned to a specific flavor, a dog chose that flavor with definite consistency over other products. A dog conditioned to liver would choose I-L over I-O. The flavored product was usually the same as the base, except that an additional flavor ingredient had been added. This same dog would discriminate for II-L, IV-L, VIII-L and other liver flavored brands against the many challenge brands tried. The dog was consistently choosing a product with only one obviously common ingredient, liver. It can thus be concluded that the dogs were actually cuing to their specific CS. As stated earlier, color cuing was eliminated by the use of dye. Residual odor had no effect because new trays were used for each sample.

No attempts have been made to determine thresholds based upon concentration of ingredients. In the case of beef, many products not displaying a flavor label contained beef. The beef conditioned dogs chose the labelled beef products over these, indicating that threshold of flavor components must be a factor. When considering another product, VI-O, it must be remembered that both chicken conditioned dogs ate VI-O over VII-C. The base product contained chicken, but was not so labelled.

Brand VII was labelled as being chicken flavored and yet dogs rejected it. Other products of this same brand were also rejected. Brand III-B was not accepted by Brownie in either Method #2 or #3 nor by Mickey on #3. Several other brands, especially of liver flavor, were intermittently accepted and rejected. Several considerations must be made as to a product's rejection. First, was the level of flavorant high enough? If not, the dog would be unable to distinguish the CS contained in the food. This would be the simple solution, but other factors must be considered. Was the food properly mixed before canning? This could account for inconsistencies in acceptance of certain brands. Was there a consistency in formulation between batches? Was the flavorant used for one product the same as that used for a like product by another company? For example, if one company used beef liver for its dog food and another porcine liver, could the dog categorize these under the same heading of liver? Likewise, what one company calls chicken may be wing tips, bones, or entrails, and give a completely different odor than the meaty part of the chicken. Another problem, especially with a bland product as beef, could be possible masking by other ingredients, such as preservatives, fortifying minerals and vitamins, or the cereal base. Since we do not know individual formulations, it is impossible to tell where our discrepancies lie.

Some very positive aspects of this work include the fact that each dog we chose conditioned to a flavor. No dog was rejected as impossible or even difficult to condition, though Lady did have difficulty with the more advanced method. One dog was considered conditioned after as few as 20 presentations. Dogs were chosen at random, with selection tending

toward the more friendly, aggressive, and good-natured ones. Becker et al. (1957) and Bayrs and Moulton (1960) concur that significant odors (food odors) elicit a better response than pure chemical odors. This may add to the probability of all dogs working well.

Random placement of the samples was followed throughout the experiment. If samples were repeatedly placed in the same location, the dogs became frustrated, acting as if they recognized the sample, but it shouldn't be there. They would then reluctantly eat the positive sample.

No position habits were developed during the trial. Many dogs developed habits in their approach to the drawer, but this didn't affect their decision.

Early in the trials, it was found that extraneous noises bothered the dogs. To remedy this situation, a radio was kept on all day, tuned to a station carrying music, to muffle secondary sounds. The dogs soon acclimated to the radio and worked well while it was playing.

At one period in the procedure, moving pictures were taken of the dogs. The camera was set up above the test area, "shooting" down into the box. The noise and cameraman disturbed some of the dogs, others paid no attention to them. Dogs showed their disturbances to the noise by looking up at the camera and not paying attention to the food samples. Two sessions were enough to acclimate them to this situation, and they worked well thereafter.

Olfaction was the primary sense used for recognition of the various flavors, although taste certainly would seem to be involved in reinforcement of the conditioning procedure. How the dogs mentally categorized these flavors is unknown. The dogs would make their selection

without touching the food but definitely sniffing it. Dogs working on Method #4 were the most obvious in their use of olfaction. They would move down the new trays, one tray at a time, sniffing at each. When they came to the CS, they either ate it or would pass by to check the other samples and then come back to it. At times the dogs would check two or three samples several times before making a choice. This could be due to the dogs inability to recognize an insufficient amount of flavor or the UCS may have confused the subject. Big'n presented a special problem. It took several seconds to arrange the food trays in the drawers, and he was so well conditioned, that he could make positive recognition before the trays were presented to him just by smelling from the other side. Olfaction was very definitely the method of discrimination.

SUMMARY

Eight dogs were used to experimentally answer the question of whether dogs can or cannot identify flavors in commercially canned dog foods.

The final conditioning method used consisted of three phases, the habituation-training phase, the conditioning-challenge phase, and the testing and reinforcement phase. In this procedure, the dog was conditioned to a flavor by letting him smell it for identification and then eat it as a gustatory reward and reinforcement. The dog was presented with two trays, one containing the conditioned stimulus (CS), the other being another flavor product or one for which no specific flavor was designated. If the dog attempted eating the negative sample, both trays were retracted and the dog received nothing. They soon learned to eat only the CS. When on trial, a variety of CS products were used, challenged by the remaining flavorants and bases negative to the specific subject. The dog food was purchased from local supermarkets so that the test foods were the same as the consumer would purchase.

The test was expanded to a method that required the dog to discriminate the CS from among five challenge flavors.

All dogs worked well for both methods, maintaining high efficiency levels throughout the experiment. One dog, however, could only contemplate four samples at a time and would "give up" when presented with six trays.

If an unidentifiable CS was presented, most dogs would not eat rather than make an error by eating an unconditioned stimulus. Though

errors were made, a dog would usually make no choice rather than a wrong choice.

Since the CS product for a particular subject had one ingredient in common, it can be assumed that the dogs were cuing on the specific flavor to which they were conditioned. If that flavor was not discernible, it may have been due to insufficient quantity, improper mixing of the flavorant with the food before canning, a masking of the flavor by other ingredients, or inconsistency among producers as to what constitutes a specific flavor.

Olfaction was definitely the process by which dogs recognized the CS. This method could be modified and utilized for threshold studies of other flavorants or odor products not associated with food. Communication has been established by this technique between the canine subjects and the researcher.

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APPENDIX

TABLE I

A total of ten brands were used with a variety of 22 different products.

BRANDS AS CODED

I F

I L

I O

VIII L

VIII B

VIII C

II L

II O

IX B

IX C

IX L

III B

III F

X F

IV L

IV O

V O

VI C

VI O

Brand Roman Numeral

Base O

Beef B

Chicken C

Fish F

Liver L

VII L

VII C

VII O

TABLE II

This table shows the data sheet for one day's session of Method #3 with Spike who was conditioned to fish. Location of the trays in the drawer are shown; the circle indicates which of the food samples was chosen. Remarks were written on the bottom of the sheet when necessary.

Dog Spike

Date Nov. 12, 1965

	1	2	3	4	5	6	7	8	9	10	11	12
Right	<div><div>I</div><div>F</div></div>	<div><div>I</div><div>F</div></div>	<div><div>III</div><div>B</div></div>	<div><div>I</div><div>F</div></div>	<div><div>I</div><div>O</div></div>	<div><div>IV</div><div>L</div></div>	<div><div>III</div><div>F</div></div>	<div><div>VII</div><div>O</div></div>	<div><div>I</div><div>F</div></div>	<div><div>I</div><div>F</div></div>	<div><div>II</div><div>O</div></div>	<div><div>III</div><div>F</div></div>
Left	<div><div>I</div><div>O</div></div>	<div><div>II</div><div>O</div></div>	<div><div>III</div><div>F</div></div>	<div><div>IV</div><div>L</div></div>	<div><div>I</div><div>F</div></div>	<div><div>III</div><div>F</div></div>	<div><div>I</div><div>O</div></div>	<div><div>III</div><div>F</div></div>	<div><div>III</div><div>B</div></div>	<div><div>II</div><div>O</div></div>	<div><div>III</div><div>F</div></div>	<div><div>IV</div><div>L</div></div>

TABLE III

This table shows the data sheet for a day on Method #4. The sequence of products indicate the order in which they were presented to the subject. The circle shows which product was chosen. Roan was conditioned to chicken.

Dog Roan

Date Feb. 14, 1966

1	II L	I F	VII O	VIII L	V O	(IX) C	8	I L	V O	IV L	VI O	IV O	(IX) C
2	VII O	I F	II L	(VI) C	V O	VIII L	9	I L	IV L	V O	(VIII) C	VI O	IV C
3	VII O	(VI) C	II L	I F	V O	VIII L	10	IV L	I L	IV O	V O	VI O	(VI) C
4	(IX) C	II L	I F	V C	VII L	VII C	11	IV O	(VIII) C	IV L	VI O	V O	I L
5	II L	I F	V O	VII O	VIII L	(VIII) C	12	IV L	IV O	VI C	(VI) C	I L	IV O
6	I F	II L	V O	(VI) C	VII O	VIII L	13	VI O	IV L	V O	I L	IV O	(IX) C
7	VII O	VIII L	(VIII) C	V C	II L	I F	14	IV L	(VIII) C	I L	IV O	VI O	V O

FIGURE I

Each brand was given a code number and labeled to indicate the presence of a flavor ingredient or abase diet.

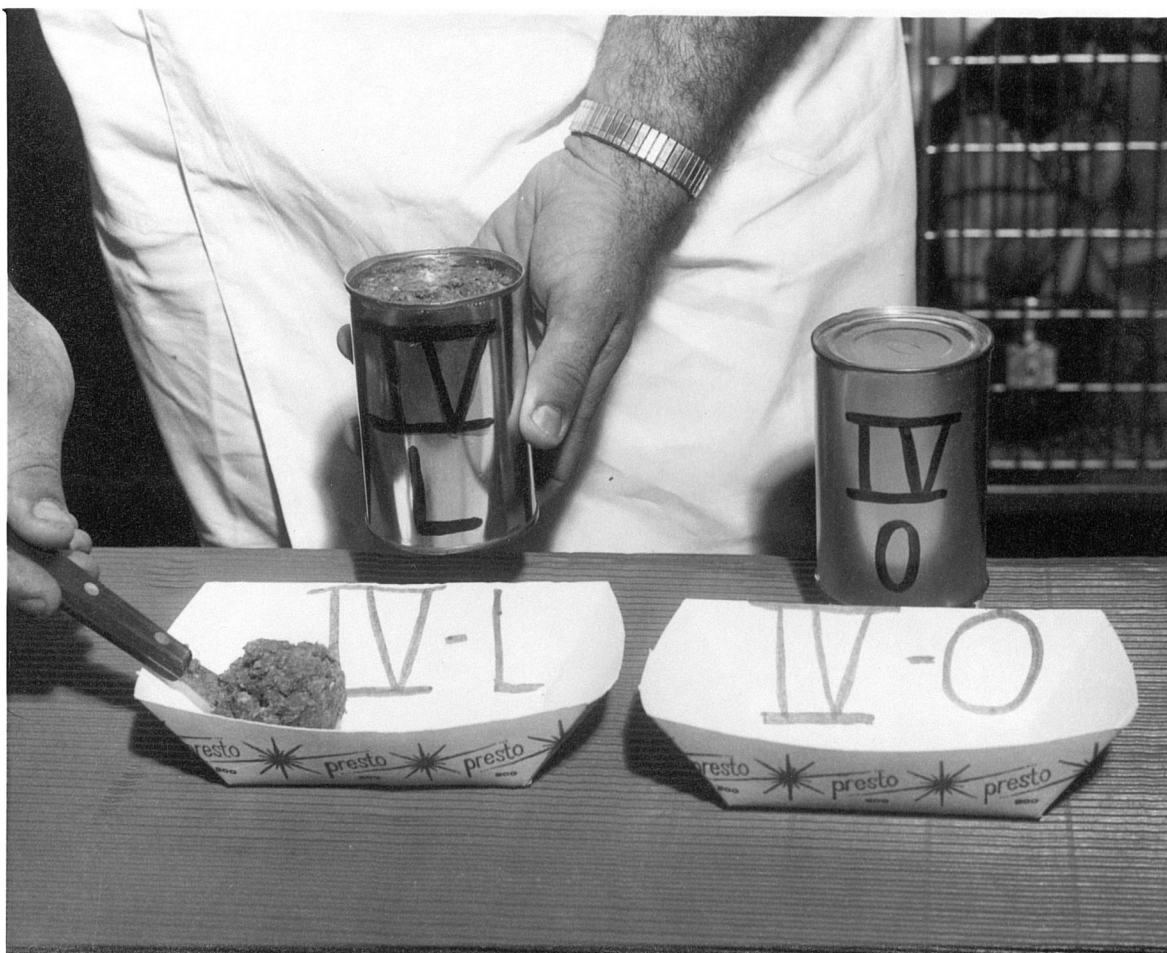


FIGURE II

The trays are ready for delivery, one containing the C.S., the other a U.C.S. Researcher can watch the subjects perform through Mirropane.



FIGURE III

When presented with the trays, the subject responds to the C.S. and eats it as additional reinforcement and reward.



FIGURE IV

Top: Drawer is delivered into the test area. Big'n is the subject and conditioned to chicken.

Bottom: Dog starts sniffing at the third tray. Note, C.S. is in the first tray.



FIGURE V

Top: Dog proceeds to tray #4.

Bottom: Subject sniffs tray #5.



FIGURE VI

Top: Dog is sniffing tray #6. Notice dogs reflection in the Mirro-
pane through which he was being observed.

Bottom: All trays thus far rejected, subject returns to trays pre-
viously sniffed.



FIGURE VII

Top: Subject returns to tray #3.

Bottom: Subject proceeds to tray #2 not before sniffed.



FIGURE VIII

Top: Subject finds C.S. in tray #1 and eats it.

Bottom: Having identified the C.S. and discriminated against the other flavorants, the subject awaits withdrawal of the drawer and presentation of a new set from which to select.



DEVELOPMENT OF A BEHAVIORAL TECHNIQUE TO ANALYZE
A DOG'S ABILITY TO DISCRIMINATE FLAVORS IN
COMMERCIALLY CANNED DOG FOODS

by

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AN ABSTRACT OF A MASTER'S THESIS

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MASTER OF SCIENCE

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Dog food companies have for several years labeled canned dog foods as containing a specific flavor. The validity of such labeling was questioned by food control officials; can the canine consumer of such products truly identify the flavors as specified? This, in essence, was the question we were to answer but of greater importance to us, was the question, "By what method can such information be obtained?"

It was proposed that if a dog could be conditioned to recognize, by olfaction, a specific flavor and give a trained response, a communication method could be established between the researcher and the canine subjects as they made positive identification of their conditioned stimulus.

Two methods were attempted but discarded due to the general inefficiency of both methods. A third method consisted of three basic phases. First, a habituation phase during which the dog was placed in the problem box and fed the conditioning stimulus flavor (CS). Once acclimated to the box and showing no apprehension about eating the CS, phase two was started. This was the conditioning-challenge phase. The dog was presented with the CS and a sample of food containing another flavor or no specific flavor. The subject had to discriminate between the two by using his sense of smell and eat the CS as a reward for having made a positive identification. If an incorrect choice was made, the food was retracted and the dog was unable to eat either sample. When a subject made positive identification for ten consecutive trials, phase three was initiated. Phase three, or the testing and reinforcement phase, was the actual test period during which several brands of CS products were used and challenged by other flavors or base products.

This method was further expanded to make the dogs choose the CS from among five challenge products.

Eight dogs were used for testing, a male and female for each of four flavors, liver, beef, chicken, and fish. All dogs conditioned rapidly and maintained high efficiency levels during phase three. Since the only common ingredient in conditioning stimuli for one dog was a given flavorant, it can be assumed that the dogs were actually cuing on flavor and nothing else.

The multiple method showed equally good results with all but one dog who could comprehend no more than four samples per trial.

If a flavor labeled product was not identified, several factors may be involved. One, the flavor product was different; two, other ingredients in the product masked the flavor; three, the flavorant was not properly blended in the food so that it was missed in some samples; or, four, the amount was simply of insufficient quantity to be detected.

Dogs can, however, identify flavored dog foods commercially available in this area.